REMARKS

Applicants respectfully request reconsideration of the application in view of the attached Declaration under 37 C.F.R. § 1.132 and Remarks.

Rejection under 35 U.S.C. § 103(a)

Claims 1, 3, 4, 9, and 10 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Lurquin, et al. (Analytical Biochemistry 65: 1-10, 1975) in view of Vosbeck, et al. (JBC 248(17): 6029-6034, 1973 and further in view of Werner, et al. (Plant Molecular Biology Reporter 16: 295-299, 1998).

Lurquin, et al. teach isolation of DNA using 2M NaCl. Vosbeck, et al. teach inactivation of pronase at 90°C. Although Lurquin, et al. teach isolation of nucleic acid using a high salt concentration (2 M), neither Lurquin, et al. nor Vosbeck teach "amplifying an object DNA from the fraction containing nucleic acid acids by PCR" as per present claim 1 (Amendment filed March 26, 2009). Werner, et al. was newly cited in the Final Office Action of June 4, 2009 to address the amendment.

Werner, et al. describe a method for determining the plus or minus mating type of *Chlamydomonas reinhardtii* by PCR with mt+ and mt- specific primers.

However, the method of Werner, et al. is a method in which an electrophoresis is performed for products amplified by PCR with the specific primers. Werner is not directed to a method of isolating nucleic acids but only briefly describes addition of Chelex 100® to *Chlamydomonas* cells in the "Material and Methods" section on page 296.

Chelex 100® is a chelating resin in which the polar resin beads bind polar cellular components after breaking open cells. Non-polar nuclear DNA and RNA remain in water solution above the resin. The 2 M NaCl-containing buffer of Lurquin, et al. is not equivalent to the chelating resin of Werner, et al. Accordingly, there is no reason to combine Vosbeck and Lurquin with Werner.

Furthermore, as previously argued, Lurquin teaches adding NaCl to a final concentration of 2 M ***after*** the heating step rather than ***before*** the heating step as required by claim 1. It is critical to bring the sample to a salt concentration of 0.5 to 2 M ***before heating*** so that histone proteins bound electrostatically to nucleic acids may be dissociated.

In the present invention, nucleic acids dissociate slowly from histone proteins since a high concentration of salt is added ***before heating***. After heating, histone proteins, which are already separated from nucleic acids, degenerate and aggregate and can be separated from nucleic acids.

On the other hand, in Lurquin, a high concentration of salt is added ***after heating*** causing aggregation and complex formation with nucleic acids included therein. If high concentration of salt is added after heating, nucleic acids cannot be separated from histone proteins. Accordingly, the method taught by Lurquin would not be effective to remove "PCR inhibitory substances" for "amplifying an object DNA from the fraction containing nucleic acid acids by PCR" as claimed.

The Examiner's position is that it was *prima facie* obvious to include a heating step in the method of Lurquin, et al. after addition of the NaCl to a final concentration of 2M to the saline/EDTA/surfactant buffer of Lurquin, et al. (Final Office Action, page 4, last paragraph) even though Lurquin, et al. only teach a heating step before addition of salt and only to 37°C, not to 80 to 100°C as claimed However, the Examiner posits that it was obvious to add a heating step after addition of salt in order to inactivate pronase as Vosbeck, et al. teach inactivation of pronase at 90°C The Examiner states that one of ordinary skill in the art would add a heating step in order to inactivate pronase.

Applicants do not teach addition of pronase at all and there is no teaching of inactivation of pronase in Lurquin, et al. Lurquin, et al. teach addition of pronase and then addition of high salt as discussed above. However, if one were to add a heating step in order to inactivate pronase as suggested by the Examiner, it seems likely that such step would be added immediately after the incubation with pronase, which would still be ***before*** addition of the high salt, not ***after*** contrary to the suggestion by the Examiner. Therefore, even assuming that one of ordinary skill in the art might reasonably heat the salt-containing buffer of Lurquin, et al. to inactivate pronase, the combination of references does not teach the claimed invention.

Furthermore, Applicants submit herewith a Declaration under 37 C.F.R. § 1.132 of Satoshi Majima (formerly Satoshi Hashiguchi), one of the inventors. The side-by-side comparison presented in the Declaration clearly shows the advantage of high salt in DNA extraction for purposes of PCR. In the Declaration, DNA was isolated in the absence of high salt concentration (item 7) and in the presence of high salt concentration (item 8) The time for these

DNA isolations was the same (10 minutes). The isolated DNAs were amplified using PCR (item 9) and used for melting curve analysis (item 10, Table 1). By the use of high salt according to the claimed method, the isolated DNA was sufficiently purified and concentrated so that the amplified DNA provided a sharp peak for Tm analysis. The DNA isolated using 0.1M NaCl (low concentration) was not sufficient for Tm analysis.

Accordingly, by the use of high salt according to the claimed method, a fast, efficient DNA isolation can be performed which can be amplified using standard techniques and used for Tm analysis. The increase in salt concentration produces a dramatic difference in result which was not expected from the cited references.

In view of Applicants' arguments and the Majima Declaration, reconsideration and withdrawal of the above ground of rejection is respectfully requested.

Rejection under 35 U.S.C. § 103(a)

The Examiner has rejected claim 2 as being unpatentable over Lurquin, et al. (Analytical Biochemistry 65: 1-10, 1975) in view of Vosbeck, et al. (JBC 248(17): 6029-6034, 1973 and further in view of Wilson, et al. (US Patent No. 7,045,679 B1). The Examiner asserts that it would have been obvious to one of ordinary skill in the art to substitute Triton X-100 for sodium sarcosylate when practicing the method of Lurquin and Vosbeck (discussed above). However, since claim 2 depends from claim 1, which is neither taught nor suggested by Lurquin and Vosbeck as discussed above, the invention defined in claim 2 is also patentably distinguished from the references, alone or in combination. Applicants respectfully request the withdrawal of the rejection.

Rejection under 35 U.S.C. § 103(a)

Claims 1-5, 9 and 10 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Burdick, et al. (EP 0 393 744 A1) in view of Akane, et al.(Biotechniques 16(2): 235, 237, 238, 1994).

Neither Burdick nor Akane teach the high salt concentrations of Applicants' claims. While the Examiner has maintained that the salt concentration is a matter of routine optimization, and that Applicants have not demonstrated criticality, Applicants address these

points with the attached Declaration of under 37 C.F.R. § 1.132 of Satoshi Majima (formerly Satoshi Hashiguchi), one of the inventors. The side-by-side comparison presented in the Declaration clearly shows the advantage of high salt in DNA extraction for purposes of PCR. In the Declaration, DNA was isolated in the absence of high salt concentration (item 7) and in the presence of high salt concentration (item 8) The time for these DNA isolations was the same (10 minutes). The isolated DNAs were amplified using PCR (item 9) and used for melting curve analysis (item 10, Table 1).

By the use of high salt according to the claimed method, the isolated DNA was sufficiently purified and concentrated so that the amplified DNA provided a sharp peak for Tm analysis. The DNA isolated using 0.1M NaCl (low concentration) was not sufficient for Tm analysis.

Accordingly, by the use of high salt according to the claimed method, a fast, efficient DNA isolation can be performed which can be amplified using standard techniques and used for Tm analysis. The use of the high salt concentration as claimed is critical to achieve this result, i.e. "amplify[ication] of the object DNA from the fraction containing nucleic acid by PCR", as claimed. Furthermore, the increase in salt concentration produces a dramatic difference in result which is unexpected in view of the prior art. Applicants believe that the attached Declaration demonstrates criticality for the claimed salt concentration (0.5-2M) and a result that was unexpected.

Response to arguments

The Examiner's position seems to be that since one of ordinary skill in the art would optimize result effective variables, one of ordinary skill in the art would increase salt concentration for the DNA isolation and then, knowing that high salt would inhibit the PCR reaction, dilute the sample before performing PCR. However, it is difficult to understand why one of ordinary skill in the art would increase salt concentration to the point where DNA polymerase would be inhibited for the purpose of PCR. By using "routine optimization" as relied upon by the Examiner, the salt concentrations as claimed would be avoided. It seems unlikely that one of ordinary skill in the art would increase the salt concentration knowing that the sample would then need to be diluted, thus lowering sensitivity.

Dilution of the sample to lower salt concentration leads to a lower DNA concentration. An advantage of the claimed method is that a small sample can be used as discussed in the specification at page 11, last paragraph. However, if the purified nucleic acids must be diluted to remove the salt, then larger sample size must be used to compensate. Thus, there is no incentive to use high salt to remove protein material as small sample volume cannot then be maintained and it is unlikely that one of ordinary skill in the art would resort to high salt for removal of protein knowing that the high salt concentration would later be troublesome for PCR and that dilution of the sample would be needed.

In view of Applicants' arguments and the Majima Declaration, reconsideration and withdrawal of the rejection is respectfully requested.

No Disclaimers or Disavowals

Although the present communication may include alterations to the application or claims, or characterizations of claim scope or referenced art, Applicant is not conceding in this application that previously pending claims are not patentable over the cited references. Rather, any alterations or characterizations are being made to facilitate expeditious prosecution of this application. Applicant reserves the right to pursue at a later date any previously pending or other broader or narrower claims that capture any subject matter supported by the present disclosure, including subject matter found to be specifically disclaimed herein or by any prior prosecution. Accordingly, reviewers of this or any parent, child or related prosecution history shall not reasonably infer that Applicant has made any disclaimers or disavowals of any subject matter supported by the present application.

CONCLUSION

In view of the foregoing Remarks and the Declaration of Satoshi Majima, it is respectfully submitted that the present application is in condition for allowance. Should the Examiner have any remaining concerns which might prevent the prompt allowance of the application, the Examiner is respectfully invited to contact the undersigned at the telephone number appearing below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

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Dated: Sept. 1, 2009

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